PCT WORLD INTELLECTUAL PROPE International Bu

REFERE E BN

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/42, 31/545, 31/43 — A1

(11) International Publication Number: **WO 94/16696**

(43) International Publication Date: 4 August 1994 (04.08.94)

(21) International Application Number: PCT/GB94/00124

(22) International Filing Date: 21 January 1994 (21.01.94)

(30) Priority Data:

9301250.8	22 January 1993 (22.01.93)	GB
9302908.0	13 February 1993 (13.02.93)	GB

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentfort, Middlesex TW8 9EP (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SMITH, Gillian, Marjory [GB/GB]; SmithKline Beecham Parmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ (GB). THORBURN, Christine, Elaine [GB/GB]; SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ (GB). ABBOTT, Karen, Hazel [GB/GB]; SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ (GB). BROWN, Tim, Neil [GB/GB]; 28 Brixham Crescent, Ruislip Mannor, Middlesex HA4 8TX (GB).

(74) Agent: WALKER, Ralph, Francis; SmithKline Beecham, Corporate Intellectual Property, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ (GB).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PHARMACEUTICAL FORMULATIONS COMPRISING CLAVULANIC ACID ALONE OR IN COMBINATION WITH OTHER BETA-LACTAM ANTIBIOTICS

(57) Abstract

A method of use of clavulanate to enhance the antibacterial activity of an antibacterial compound against microorganisms having an antibiotic resistance mechanism other than β-lactamase enzyme mediated resistance. Pharmaceutical formulations and methods of use which exploit this method.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

PHARMACEUTICAL FORMULATIONS COMPRISING CLAVULANIC ACID ALONE OR IN COMBINATION WITH OTHER BETA-LACTAM ANTIBIOTICS

This invention relates to pharmaceutical formulations, in particular to novel formulations for the treatment of bacterial infections.

Clavulanic acid is a known compound having efficacy in inhibiting bacterial β-lactamase enzymes, which degrade β-lactam antibiotics such as penicillins and confer resistance to such antibiotics. Clavulanic acid in the form of its derivatives (hereinafter termed "clavulanate"), particularly its salts, are consequently used in formulations in combination with β-lactam antibiotics, for example as described in GB 2005538, to suppress the activity of β-lactamase enzymes which mediate bacterial resistance to β-lactam antibiotics.

The possibility of clavulanic acid inhibiting bacterial resistance mechanisms other than β-lactamase-mediated has been suggested, e.g. D. Greenwood, Proc. Ist. Symp. Augmentin: Excerpta Med. Int. Cong. Ser. (1980) 80-83. This publication only describes *in vitro* work on *E. coli*, and the β-lactam antibiotics cephalexin, cephadrine, and mecillinam. This publication concludes that the observed effect is unlikely to be of any general therapeutic significance.

The inventors have discovered an unexpected further activity of clavulanic acid in enhancing the effectiveness of antibacterial compounds against microorganisms which have an antibiotic resistance mechanism which is different to that mediated by β-lactamase enzymes. This effect is observed *in vivo*, and may be of therapeutic significance in the therapy of infections by β-lactamase negative penicillin resistant pathogens such as *S. pneumoniae* and *H. influenzae*.

Accordingly the present invention provides the use of clavulanate to enhance the antibacterial activity of antibacterial compounds against microorganisms having an antibiotic resistance mechanism other than β-lactamase enzyme mediated resistance.

The present invention also provides a method of use of clavulanate in the manufacture of a medicament formulation for the treatment of infection of humans or animals by microorganisms having a resistance mechanism other than β-lactamase mediated resistance.

The present invention further provides a pharmaceutical formulation comprising clavulanate, for use as an active therapeutic substance in the treatment of infection of humans or animals by microorganisms having a resistance mechanism other than β-lactamase

mediated resistance.

Further the invention provides a method for the treatment of an infection by microorganisms having a resistance mechanism other than β-lactamase mediated resistance in humans or animals, which comprises administering thereto clavulanate.

The above uses, formulation and methods are particularly suitable in respect of penicillin-resistant microorganisms, e.g. which are believed to have a penicillin-binding-protein (hereinafter termed "PBP") mediated resistance mechanism, although the exact mechanism of the resistance mechanism inhibited by clavulanate in the present invention is not known, and the invention is not limited to any specific mechanism.

This type of microorganism includes penicillin-resistant organisms such as *Streptococcus spp.*, e.g. *S. pneumoniae*, *Haemophilus spp., e.g. H. influenzae, Staphylococcus spp.*, *Enterococcus spp.*, and *Neisseria spp.*, e.g. *N. gonococcus* and *N. meningitidis*. Of these, *S. pneumoniae* and *H. influenzae* are major pathogens.

Typically the clavulanate may be present as a salt, preferably as its potassium salt, ie potassium clavulanate. The clavulanate may in some cases be antibacterially effective by itself against the organisms, but preferably the clavulanate is used in the above uses, formulations and methods of this invention in combination with an antibacterial agent, for example an antibiotic, suitably a β-lactam antibiotic such as a penicillin or cephalosporin. Preferably the antibacterial agent is a β-lactam antibiotic.

Suitable β-lactam antibiotics include the penicillins: amoxycillin, ampicillin, apalcillin, aspoxicillin, azidocillin, azlocillin, aztreonam, benzylpenicillin, bacampicillin, carbenicillin, cloxacillin, cyclacillin, dicloxicillin, epicillin, flucloxacillin, lenampicillin, mecillinam, methicillin, mezlocillin, phenoxymethylpenicillin, piperacillin, pivampicillin, propicillin, sulbenicillin, talampicillin, and ticarcillin; and the cephalosporins: cefaclor, cefadroxil, cefatrizine, cefclidine, cefamandole, cefazolin, cefbuperazone, cefcanel daloxate, cefdinir, cefepime, cefetamet pivoxil, cefixime, cefminox, cefminoxime,. cefmetazole, cefonicid, cefoperazone, cefotaxime, cefotetan, cefotiam, cefotiam hexetil, cefoxitin, cefpimizole, cefpiramide, cefpirome, cefpodoxime proxetil ,cefprozil, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime axetil, cefuroxime, cephacetrile, cephalexin, cephaloridine, cephalothin, cephamanadole nafate, cephapirin, cephoperazone, cefsulodin, cefuzonam, cephradine, loracarbef, DQ 2556, ME1207, S-1006, SCE-2787 and moxalactam.

Particularly suitable β-lactam antibiotics include the penicillins: amoxycillin, ampicillin, apalcillin, aspoxicillin, azidocillin, azlocillin, aztreonam, benzylpenicillin, bacampicillin, carbenicillin, cloxacillin, cyclacillin, dicloxicillin, epicillin, flucloxacillin, lenampicillin, mecillinam, methicillin, mezlocillin, phenoxymethylpenicillin, piperacillin, pivampicillin, propicillin, sulbenicillin, talampicillin, and ticarcillin; and the cephalosporins: cefaclor, cefadroxil, cefatrizine, cefclidine, cefamandole, cefazolin, cefbuperazone, cefcanel daloxate, cefdinir, cefepime, cefetamet pivoxil, cefminox, cefminoxime, cefmetazole, cefonicid, cefoperazone, cefotaxime, cefotetan, cefotiam, cefotiam hexetil, cefoxitin, cefpimizole, cefpiramide, cefpirome, cefprozil, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cephacetrile, cephalexin, cephaloridine, cephalothin, cephamanadole nafate, cephapirin, cephoperazone, cefsulodin, cefuzonam, cephradine, DQ 2556, ME1207, S-1006, SCE-2787 and moxalactam.

The use of clavulanate together with certain of the above-listed β-lactam antibiotics is believed to be novel *per se*, and therefore in a further aspect of this invention there is provided a pharmaceutical formulation comprising in combination clavulanate together with a cephalosporin antibiotic selected from the cephalosporins: cefaclor, cefaclidine, cefcanel daloxate, cefetamet pivoxil, cefminox, cefodizime, cefpimizole, cefpiramide, cefpodoxime proxetil, cefprozil, cefuzonam, DQ 2556, ME 1207, S-1006, SCE-2787 and loracarbef.

Suitable examples of this last-mentioned formulation include in combination clavulanate together with a cephalosporin antibiotic selected from the cephalosporins cefaclor and cefprozil

The present invention also provides this last-mentioned formulation, e.g clavulanate in combination with cefaclor or cefprozil, for use as an active therapeutic substance in the treatment of infection of humans or animals by microorganisms.

The invention therefore further provides the use of clavulanate in combination with with a cephalosporin antibiotic selected from this last-mentioned list, e.g clavulanate in combination with cefaclor or cefprozil, in the manufacture of a medicament for the treatment of bacterial infections.

The invention also further provides a method of treatment of an infection by microorganisms in humans or animals, which comprises administering thereto clavulanate and a cephalosporin antibiotic selected from this last-mentioned list, e.g clavulanate in combination with cefaclor

or cefprozil.

The novel formulations, uses and methods of this further aspect of the invention may be effective against β-lactamase negative penicillin resistant pathogens such as *S. pneumoniae* and *H. influenzae*, as discussed above, and may also be effective against other organisms including β-lactamase positive strains of *N. gonorrhoeae*, *Staphylococcus* spp. (e.g *S.aureus*), *Bacteroides fragilis*, *Moraxella catarrhalis*, *Escherichia coli* and *Klebsiella pneumoniae*, for example in otitis media, urinary tract infections, respiratory tract, skin and soft tissue infections.

The β-lactam antibiotics referred to herein may be in the form of the free acids or pharmaceutically acceptable salts or in-vivo hydrolysable esters.

Preferred antibacterial agents include amoxycillin, suitably in the form of amoxycillin trihydrate for oral use, and in the form of sodium amoxycillin for parenteral use, and the cephalosporins cefaclor or cefprozil.

The clavulanate and any other antibacterial agent such as the penicillin or cephalosporin antibiotics, as used in this invention, whether in the form of the free acids, salts, esters or derivatives thereof are preferably each in a substantially pure form, e.g. at least 60% pure, more suitably at least 75% pure, preferably at least 85% especially at least 98% pure on a weight basis.

In the methods of treatment of this invention, clavulanate and an antibacterial agent such as the penicillin or cephalosporin antibiotics, e.g amoxycillin, cefaclor or cefprozil, may be administered together, simultaneously, successively or in any order, but typically may be administered together as a co-formulation.

The formulation may be formulated for administration by any route, such as oral, topical or parenteral. The route of choice may for example be determined by the route of choice for the antibacterial agent used in combination with the clavulanate. The formulation may be in the form of tablets, capsules, powders, granules, lozenges, creams or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

The topical formulations of the present invention may be presented as, for instance, ointments, creams or lotions, eye ointments and eye or ear drops, impregnated dressings and aerosols, and may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams.

The formulations may also contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions. Such carriers may be present at from about 1% up to about 98% of the formulation. More usually they will form up to about 80% of the formulation.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. Such tablets may also include an effervescent couple of generally known type, e.g a solid carboxylic acid and an alkali metal carbonate or bicarbonate. Such tablets may also include a chewable base such as mannitol, sorbitol or lactose, optionally together with an effervescent couple, for example as described in EP 0389177. Such tablets and solid dosage forms may be made by any of the generally known methods for such dosage forms, and may be coated according to methods well known in normal pharmaceutical practice.

Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and, if desired, conventional flavouring or colouring agents.

Suppositories will contain conventional suppository bases, e.g. cocoa-butter or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilizing clavulanate and any antibacterial agent and a sterile vehicle, water being preferred. These active compounds, depending on the vehicle and concentration used, can be either suspended or dissolved in

the vehicle. In preparing solutions the active compounds can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing.

Advantageously, agents such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the formulation can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilized powder is then sealed in the vial and an accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions are prepared in substantially the same manner except that the ingredients of the suspension are suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The formulation can be sterilised by exposure of its dry constituents to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the formulation to facilitate uniform distribution of the active compounds.

Since salts of clavulanic acid are extremely hygroscopic the solid and non-aquous liquid formulations of this invention must be prepared in dry conditions, typically at a relative humidity of 30% or less. All constituents of formulations of this invention should be predried. Aqueous solution and suspension formulations of this invention can only be provided in the form of dry solids for make up into aqueous solution or suspension shortly prior to use, for example 5 days in the case of oral suspensions. It may also be necessary to maintain such suspensions at low temperatures, e.g >5^{o}C.

In view of the extreme moisture sensitivity of clavulanate, aqueous suspensions or solutions insofar as they contain clavulanate must be provided as dry solids for reconstitution with water shortly before administration.

A formulation according to the invention may be in unit dosage form, for example unit dosage form for oral or parenteral administration, which latter will primarily include administration by injection or infusion, especially intramuscular and intravenous administration.

The above-mentioned formulations may contain 0.1 - 90% by weight, preferably from 10 - 60% by weight of the active materials, depending on the method of administration.

The clavulanate may suitably be administered to the patient at a daily dosage of from 0.3 to 15 mg/kg, preferably from 0.7 to 10 mg/kg, for

example from 0.7 to 7 mg/kg, of body weight. For an adult human (of approximately 70 kg body weight), from 25 to 1000 mg, preferably from 50 to 500 mg, of clavulanate expressed as its free acid equivalent may be administered daily, suitably in from 1 to 6, preferably from 2 to 4, separate doses. Higher or lower dosages may, however, be used in accordance with clinical practice.

When the formulations according to the invention are presented in unit dosage form, each unit dose may suitably comprise from 12.5 to 1000 mg, preferably from 12.5 to 250 mg, of clavulanate. Each unit dose may, for example, be 12.5, 25, 50, 75, 100, 125, 150, 200, or 250 mg of clavulanate.

The ratio of the amount of the clavulanate used according to the invention to the amount of any antibacterial agent present may vary within a wide range. In a pharmaceutical or medicament formulation of this invention the said ratio may, for example, be from 1:1 to 1:30; more particularly, it may, for example, be from 1:1 to 1:12, for example 1:2, 1:3, 1:4,1:5, 1:6, 1:7, 1:8, or 1:9 by weight, suitably within a variance of ± 10%.

Suitable unit dosages and maximum daily dosages of any antibacterial agent used in combination with clavulanate in this invention may for example be determined according to the unit dosages and maximum daily dosages of the agent used conventionally. For example amoxycillin is generally provided in unit dosages of 125 to 1000 mg, administered from 2 to 4 times daily to a typical daily dosage of 125 to 3000 mg per day. For example cefaclor is generally provided in unit dosages of 250 and 500 mg, and may be dosed up to a maximum daily dosage of 4000 mg per day.

A preferred combination of this invention is clavulanate with amoxycillin, in a ratio clavulanate : amoxycillin in the range 1:1 to 1:12, for example together in a formulation. An example of a suitable formulation according to the invention for oral administration is one comprising from 125 to 3000 mg, preferably from 500 to 1000 mg, of amoxycillin trihydrate, in admixture or conjunction with from 12.5 to 250 mg, preferably from 25 to 125 mg, of potassium clavulanate per unit dose.

A further example of a suitable formulation according to this invention for parenteral administration is one comprising from 125 to 3000 mg of sodium amoxycillin, in admixture or conjunction with from 12.5 to 250 mg, preferably from 25 to 125 mg, of potassium clavulanate.

An example of a unit dosage form of a formulation of this

invention comprises 12.5 to 1000 mg of potassium clavulanate and 62.5 to 500 mg of cefaclor.

The following examples illustrate the antibacterial activity of amoxycillin, cefaclor and cefprozil, and clavulanate in combination and compare it with the activity of amoxycillin alone against a penicillin-resistant strain of *Streptococcus pneumoniae*.

Figs. 1 to 5 show graphically the level of *S. pneumoniae* growth in vivo following administration of amoxycillin : clavulanate, cefaclor : clavulanate and cefprozil : clavulanate compared with comparisons and controls. In these examples the abbreviations NTC = non treated control, AMX = amoxycillin, CA = potassium clavulanate, CEC = cefaclor and PRO = cefprozil are used, and doses of clavulanate and the antibiotics in mg/kg are shown in the graphs against Log_{10} cfu per pair of lungs.

Example 1

Summary

A model of a penicillin-resistant *Streptococcus pneumoniae* respiratory infection in immunocompromised rats was developed for comparative efficacy studies with antibacterials. Rats were rendered neutropenic with cyclophosphamide, and infected by intrabronchial instillation of a penicillin-resistant strain of *S.pneumoniae*.

The infection persisted in the rats' lungs for at least four days at a mean count of 7.0 log 10 cfu/lungs, although the mortality rate was low. Oral therapy commenced 24 h post infection and continued q12h for three days. Assessment of therapy was by counts of bacteria recovered from lung samples at intervals during the studies.

Amoxycillin (200 mg/kg) showed little activity against this strain, but amoxycillin/clavulanate (200/100 mg/kg) was effective in reducing numbers of *S.pneumoniae* from the lungs within 48 h of therapy, and with a further significant reduction to 2-3 log 10 cfu/lungs at 72 h and 96 h.

Materials and Methods

Animals: Weanling male specific pathogen free ("SPF") rats (60-80 g, CD strain) were supplied by Charles River UK Ltd.

Induction of leukopenia: Rats were dosed intraperitoneally with 0.5 ml cyclophosphamide (Endoxana, Boehringer Ingelheim Ltd., Bracknell). at 50 mg/kg three days before, and on the day of infection.

Organism: *S.pneumoniae* N1387 was used in both studies.

Inoculum: A stock inoculum of *S.pneumoniae* N1387 (stored at -

70°C) was grown on blood agar at 37°C and the growth from six plates was suspended in 3 ml Todd Hewitt broth (TH). This was further diluted 1:5 in molten nutrient agar maintained at 40°C.

Anaesthesia: Rats were anaesthetised by separate intramuscular injections of 50µl of fentanyl fluanisone at 0.1 ml/kg (Hypnorm, Janssen Pharmaceuticals Ltd., Grove), diazepam at 0.5 mg/kg (Valium, Roche products Ltd,. Welwyn Garden City). The drugs were prepared in sterile distilled water.

Infection: Anaesthetised rats were infected by intrabronchial instillation of a 50µl inoculum containing 6-7 log 10 cfu *S. pneumoniae* by means of non-surgical intratracheal intubation.

Compounds: Amoxycillin trihydrate and potassium clavulanate (SmithKline Beecham Pharmaceuticals, Worthing) were dissolved in pH 8.0 phosphate buffer and sterile distilled water respectively.

Dosage: Groups of 5 rats received 0.5 ml of each agent by oral gavage. Therapy commenced 24 h post infection, and continued b.i.d. (q12h) for three days. Rats received amoxycillin alone at 200 mg/kg or amoxycillin/clavulanic acid at 200/100 mg/kg to give AUCs in plasma equivalent to those produced in man following a 500 mg dose of amoxycillin or a 625 mg dose of Augmentin (500 mg amoxycillin plus 125 mg potassium clavulanate) respectively.

Results.

An initial study was performed to look at the effects of amoxycillin and amoxycillin/clavulanate following two days of twice-daily therapy (q12h), and lung bacterial counts were taken at 72 h only (Figure 1). In this study, following infection with 7.0 log 10 cfu *S. pneumoniae* per rat, amoxycillin/clavulanate was effective in significantly reducing bacterial numbers by 72 h in comparison with the non-treated controls ($p=<0.05$). Following therapy with amoxycillin alone, some reduction in bacterial numbers was noted, although counts were not significantly different from those in the non-treated control group ($p=>0.5$). In this study, amoxycillin/clavulanate was significantly different from amoxycillin (p less than 0.05).

A further study was carried out in order to confirm these findings, and to determine the course of infection over a four day period. The results are shown in Figure 2. Following infection with 6.6 log 10 cfu per rat, *S. pneumoniae* grew well in the rats' lungs, reaching 7.6 ± 1.0 log 10 cfu/lungs in the non-treated group by 72 h, with 7.0 ± 0.6 log 10 cfu/lungs

persisting at 96h post infection. Amoxycillin again had some effect on the infection, and significantly reduced numbers of the organism to 5.0±0.5 by 72h, although at 96 h the counts were not significantly different from those in the non-treated control group. Amoxycillin/clavulanate, however, caused a more rapid reduction in numbers, with 4.6±1.5 log 10 cfu/lungs detectable at 48 h, and with a further reduction by 72 h-96 h with only 2.8±1.2 and 2.6±0.9 log 10 cfu/lungs detectable respectively. In this study, amoxycillin/clavulanate was again significantly more effective than amoxycillin alone ($p=<0.05$).

Discussion

These results demonstrate that, in this rat respiratory model, twice daily oral therapy with amoxycillin/clavulanate at 200/100 mg/kg (625 mg equivalent in man) was significantly more effective than amoxycillin at 200 mg/kg (500 mg equivalent in man) in reducing numbers of a penicillin- and macrolide- resistant strain of *S. pneumoniae* from the lungs of infected animals.

Example 2

A further experiment was carried out along similar lines to Example 1, again using specific pathogen-free rats. As well as therapy with potassium clavulanate : amoxycillin, therapy with potassium clavulanate : cefaclor and potassium clavulanate : cefprozil was also investigated.

As a control, amoxycillin alone and in the presence of clavulanate was tested, and no loss of active amoxycillin was detected in the site of infection, i.e. the lungs of rats treated with amoxycillin alone or with clavulanate, confirming the absence of β-lactamase-producing organisms. Synergy between amoxycillin and clavulanate was also shown using an intraperitoneal infection, which is normally a sterile site, negating the possibility of other bacteria interfering with the test.

The results of these experiments are shown graphically in Figs. 3, 4 and 5.

Fig. 3. shows that amoxycillin : clavulanate was significantly more effective than amoxycillin alone against three penicillin resistant strains of *S. pneumonia*

Fig. 4 shows that cefaclor : potassium clavulanate was significantly more effective than cefaclor alone against penicillin resistant *S. pneumoniae* N1387, showing effectiveness at cefaclor : clavulanate 200 : 50 mg/kg.

Fig. 5 shows that cefprozil : potassium clavulanate was significantly more effective than cefprozil alone against penicillin resistant *S. pneumoniae* N1387, showing effectiveness at cefprozil : clavulanate 50 : 50 mg/kg and 25 : 50 mg/kg.

Example 3.

Method.

Determinations of the minimum inhibitory concentration (MIC) were performed in agar using serial dilutions of the test compounds alone or in the presence of constant concentrations of clavulanic acid (2 or 4 µg/ml). The agar used was Mueller Hinton (BBL) supplemented with 5% lysed horse blood in the case of *H. influenzae* and *M. catarrhalis*, except for *B. fragilis* where Wilkins-Chalgren agar (Oxoid) was used. A multipoint inoculator was used to drop 1µl of undiluted culture of *S. aureus*, 10-fold dilutions of *B. fragilis, H. influenzae* and *M. catarrhalis* and 100-fold dilutions of *E. coli* and *K. pneumoniae* onto the surface of the agar, to give an inoculum of approximately 10^4 to 10^5 cfu/spot.

Results

The activities of cefaclor ("Distaclor" - Trade Mark (Dista) Lot No 64473ae (96% pfa)) alone and in the presence of clavulanic acid are illustrated in Table 1. Against β-lactamase producing strains of *S. aureus*, 2 and 4 µg/ml clavulanic acid markedly improved the activity of cefaclor, reducing the geometric mean (Gmean) MIC's from 12.44 to 1.13 and 0.73 µg/ml respectively. The activity of cefaclor against plasmid mediated β-lactamase producing strains of *E. coli* and *K. pneumoniae* was also improved by clavulanic acid, the Gmean MIC values being reduced considerably (Table 1). Against β-lactamase producing strains of *M. catarrhalis, H. influenzae* and the strains of *B. fragilis* tested, clavulanic acid at a concentration of 2 or 4 µg/ml improved the activity of cefaclor.

Table 1

Organism (No.)	treatment	range	MIC75	MIC90	Gmean
S.aureus)	CEC	1-256	32	64	12.44
P+)	CEC+CA2	0.12-8	2	4	1.13
(10))	CEC+CA4	0.25-8	2	2	0.73
E.coli)	CEC	1-32	4	32	4.32

R+)	CEC+CA2	1-4	2	4	1.85
(11))	CEC+CA4	0.5-4	2	4	1.36
K.pneumo-)	CEC	1-256	256	256	17.67
-niae R+)	CEC+CA2	0.5-256	32	256	3.28
(7))	CEC+CA4	0.25-2.56	16	256	2.00
M.catarr-)	CEC	1-8	8	8	4.88
halis)	CEC+CA2	0.06-0.5	0.25	0.25	0.23
(14))	CEC+CA4	0.008-0.25	0.008	0.25	0.01
H.influen-)	CEC	2-16	16	16	7.64
zae)	CEC+CA2	1-32	8	16	5.04
(15))	CEC+CA4	1-16	4	16	3.48
B.fragilis)	CEC	64-512	4	128	150.67
(19))	CEC+CA2	1.256	4	256	4.00
)	CEC+CA4	1-512	4	128	5.32

Claims.

1. A method of use of clavulanate to enhance the antibacterial activity of an antibacterial compound against microorganisms having an antibiotic resistance mechanism other than β-lactamase enzyme mediated resistance.

2. A method of use of clavulanate in the manufacture of a medicament formulation for the treatment of infection of humans or animals by microorganisms having a resistance mechanism other than β-lactamase mediated resistance.

3. A pharmaceutical formulation comprising clavulanate, for use as an active therapeutic substance in the treatment of infection of humans or animals by microorganisms having a resistance mechanism other than β-lactamase mediated resistance.

4. A method for the treatment of an infection by microorganisms having a resistance mechanism other than β-lactamase mediated resistance in humans or animals, which comprises administering thereto clavulanate.

5. A method or formulation according to any one of claims 1 to 4 wherein the mechanism of resistance is penicillin-binding-protein (hereinafter termed "PBP") mediated resistance mechanism.

6. A method or formulation according to any one of claims 1 to 4 wherein the microorganisms are penicillin-resistant organisms.

7. A method or formulation according to any one of claims 1 to 4 wherein the microorganisms are selected from *Streptococcus spp.*, *Haemophilus spp.*, *Staphylococcus spp.*, *Enterococcus spp.*, and *Neisseria spp.*, or *N. meningitidis*.

8. A method or formulation according to claim 7 wherein the microorganisms are *S. pneumoniae* or *H. influenzae*.

9. A method or formulation according to any one of the preceding

claims wherein the clavulanate is present as a potassium clavulanate.

10. A method or formulation according to any one of the preceding claims wherein the clavulanate is in combination with an antibacterial agent.

11. A method or formulation according to claim 10 wherein the antibacterial agent is a β-lactam antibiotic selected from the penicillins: amoxycillin, ampicillin, apalcillin, aspoxicillin, azidocillin, azlocillin, aztreonam, benzylpenicillin, bacampicillin, carbenicillin, cloxacillin, cyclacillin, dicloxicillin, epicillin, flucloxacillin, lenampicillin, mecillinam, methicillin, mezlocillin, phenoxymethylpenicillin, piperacillin, pivampicillin, propicillin, sulbenicillin, talampicillin, and ticarcillin; and the cephalosporins: cefaclor, cefadroxil, cefatrizine, cefclidine, cefamandole, cefazolin, cefbuperazone, cefcanel daloxate, cefdinir, cefepime, cefetamet pivoxil, cefixime, cefminox, cefminoxime,. cefmetazole, cefonicid, cefoperazone, cefotaxime, cefotetan, cefotiam, cefotiam hexetil, cefoxitin, cefpimizole, cefpiramide, cefrirome, cefpodoxime proxetil,. cefprozil, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime axetil, cefuroxime, cephacetrile, cephalexin, cephaloridine, cephalothin, cephamanadole nafate, cephapirin, cephoperazone, cefsulodin, cefuzonam, cephradine, loracarbef, DQ2556, ME1207, S-1006, SCE-2787 and moxalactam.

12. A method or formulation according to claim 11 wherein the β-lactam antibiotic is amoxycillin.

13. A method or formulation according to claim 11 wherein the β-lactam antibiotic is cefaclor.

14. A method or formulation according to claim 11 wherein the β-lactam antibiotic is cefprozil.

15. A pharmaceutical formulation comprising in combination clavulanate together with a cephalosporin antibiotic selected from the cephalosporins: cefaclidine, cefcanel daloxate, cefetamet pivoxil, cefminox, cefodizime, cefpimizole, cefpiramide, cefpodoxime proxetil , cefuzonam, DQ2556, ME 1207, S-1006, SCE-2787 and loracarbef.

16. A pharmaceutical formulation comprising in combination clavulanate together with cefaclor.

17. A pharmaceutical formulation comprising in combination clavulanate together with cefprozil.

18. A formulation according to claim 15, 16 or 17 for use as an active therapeutic substance in the treatment of infection by humans or animals by microorganisms.

19. The use of clavulanate together with a cephalosporin antibiotic selected from those listed in claim 15 in the manufacture of a medicament for the treatment of bacterial infections.

20. The use of clavulanate together with a cephalosporin antibiotic selected from cefaclor and cefprozil in the manufacture of a medicament for the treatment of bacterial infections.

21. A method of treatment of an infection by microorganisms in humans or animals, which comprises administering thereto clavulanate and a cephalosporin antibiotic selected from those listed in claim 15.

22. A method of treatment of an infection by microorganisms in humans or animals, which comprises administering thereto clavulanate and a cephalosporin antibiotic selected from cefaclor or cefprozil.

Counts of viable bacteria isolated at 72 h from rats infected with S. pneumoniae N1387

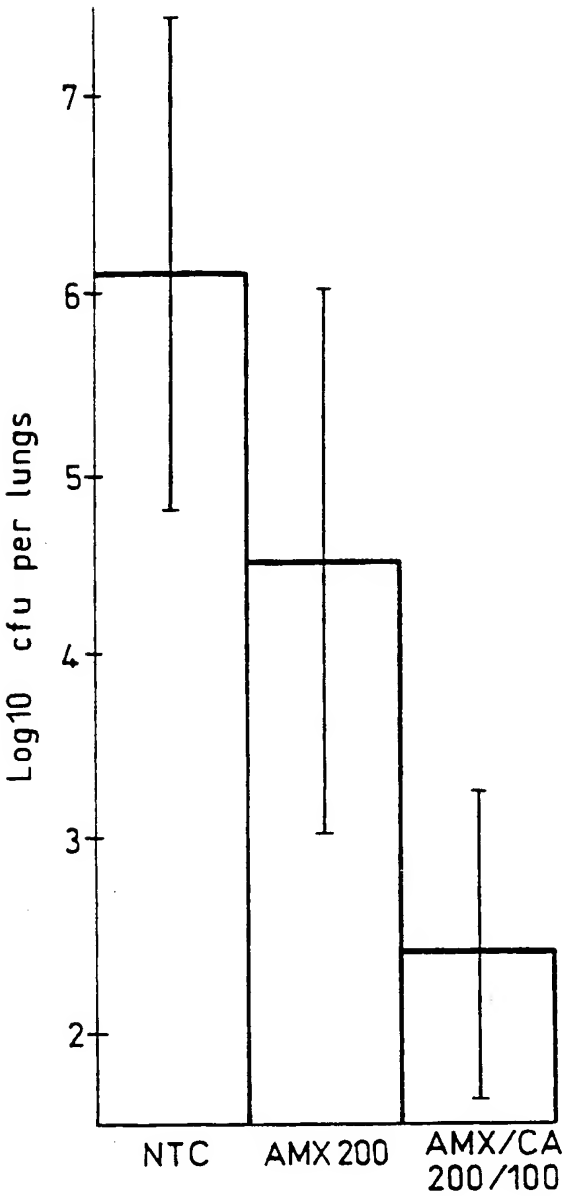

Fig. 1

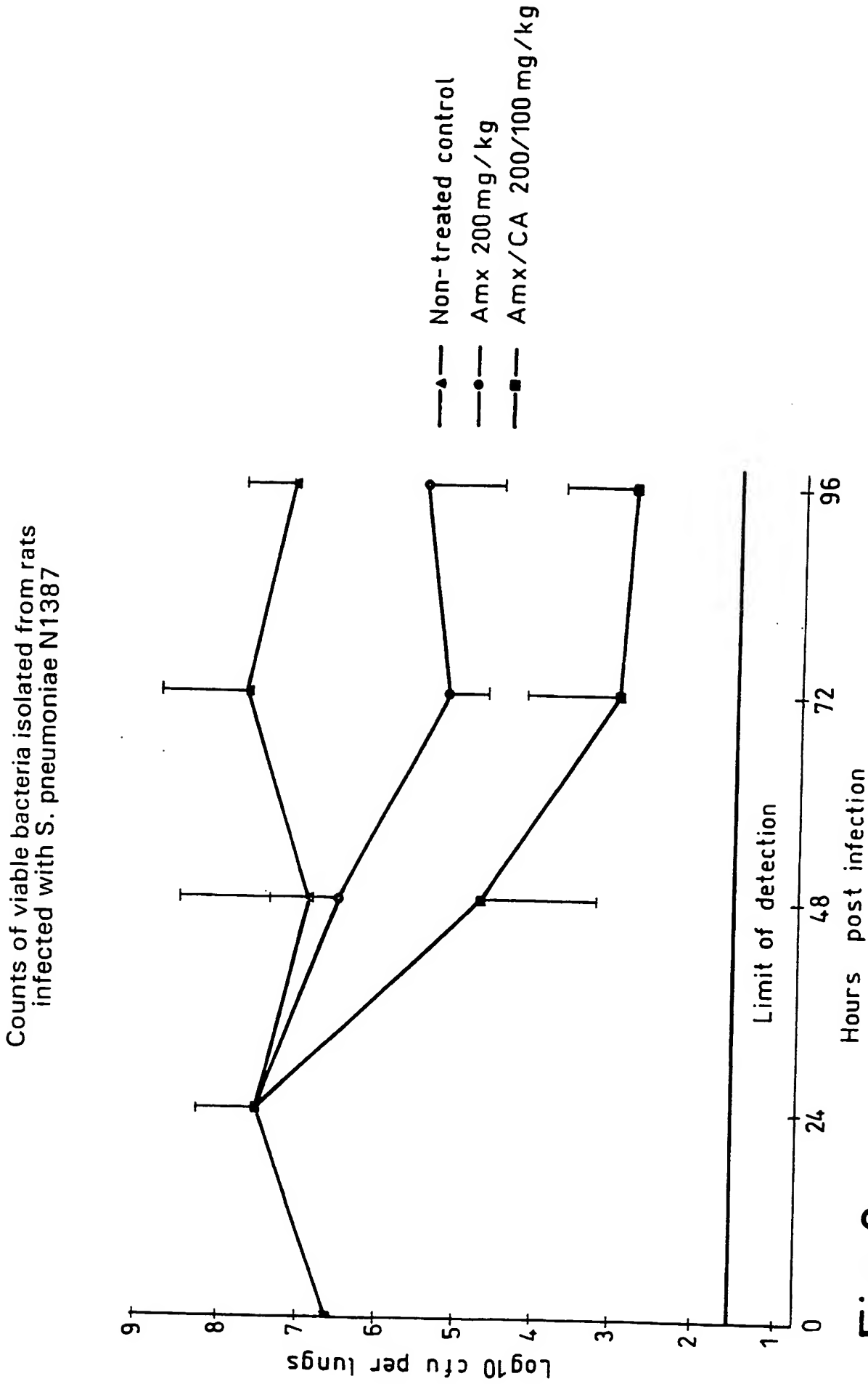
Counts of viable bacteria isolated from rats infected with S. pneumoniae N1387
Non-treated control
Amx 200mg/kg
Amx/CA 200/100 mg/kg
Log10 cfu per lungs
9
8
7
6
5
4
3
2
1
Limit of detection
0
24
48
72
96
Hours post infection

Fig. 2

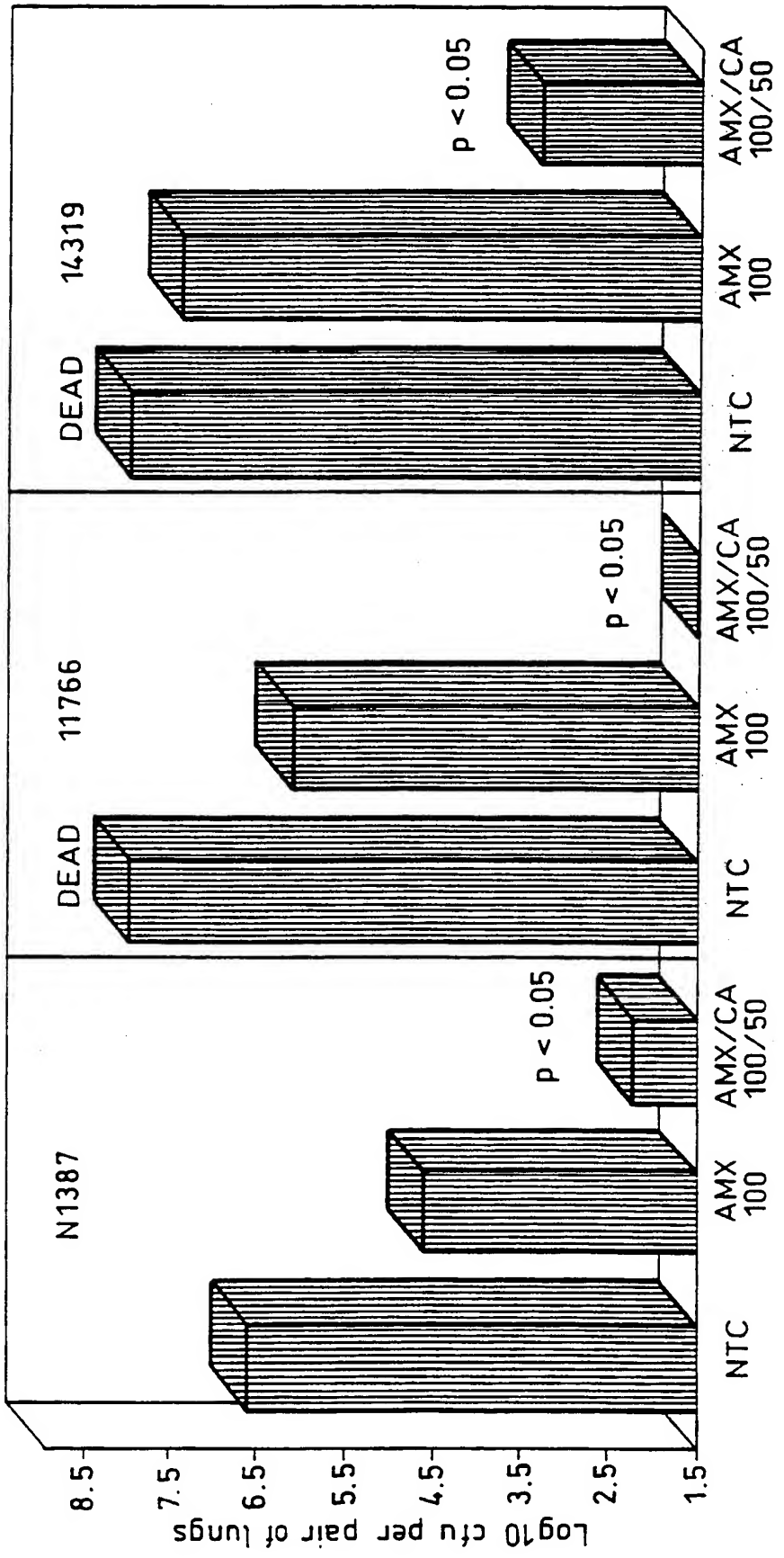
Mean lung viable counts from rats infected with strains of S. pneumoniae
Log10 cfu per pair of lungs
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
N1387
DEAD
11766
DEAD
14319
NTC
AMX 100
AMX/CA 100/50
p < 0.05

Fig. 3

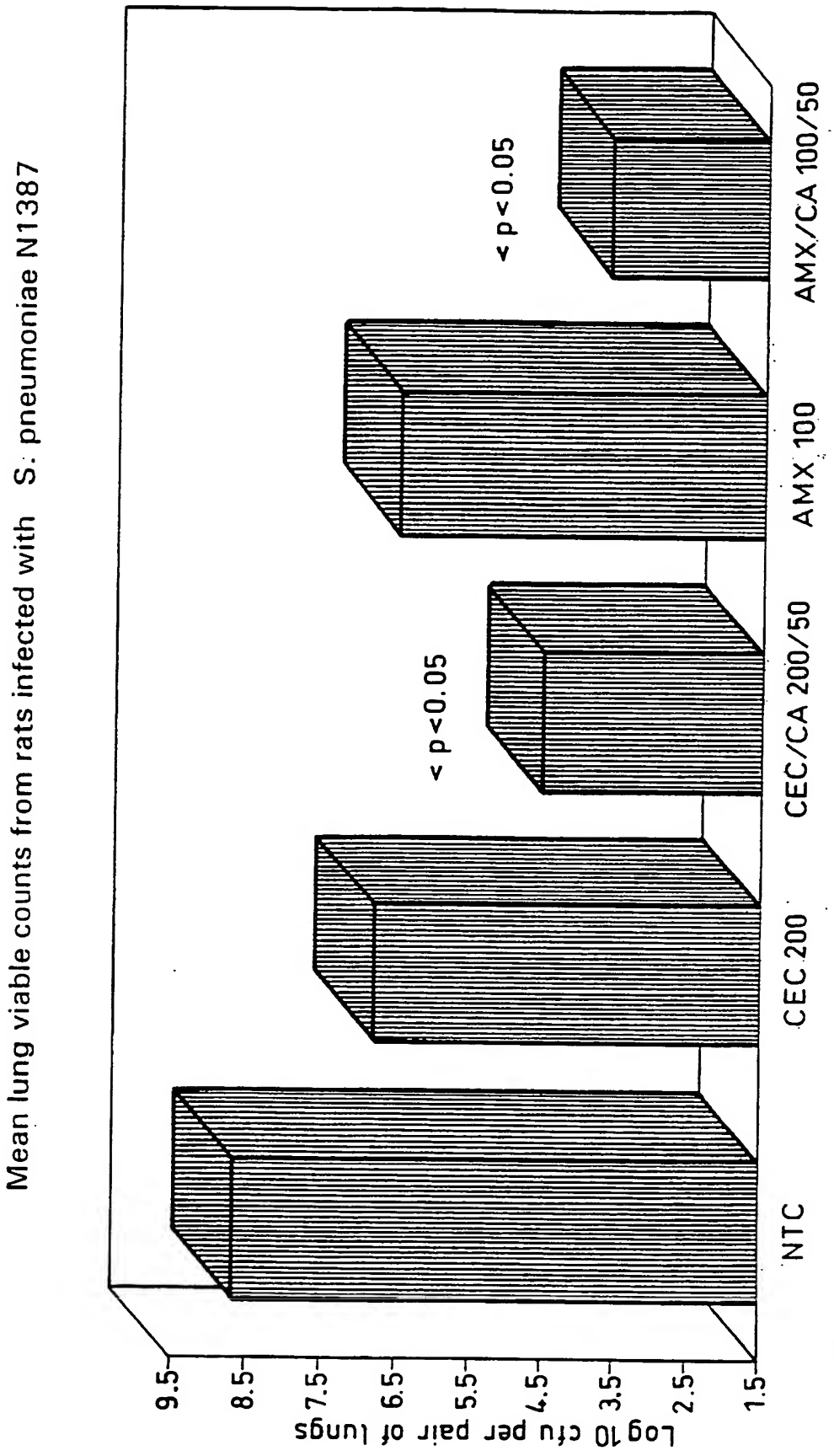
Mean lung viable counts from rats infected with S. pneumoniae N1387
Log10 cfu per pair of lungs
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
NTC
CEC 200
CEC/CA 200/50
AMX 100
AMX/CA 100/50
< p<0.05
< p<0.05

Fig.4

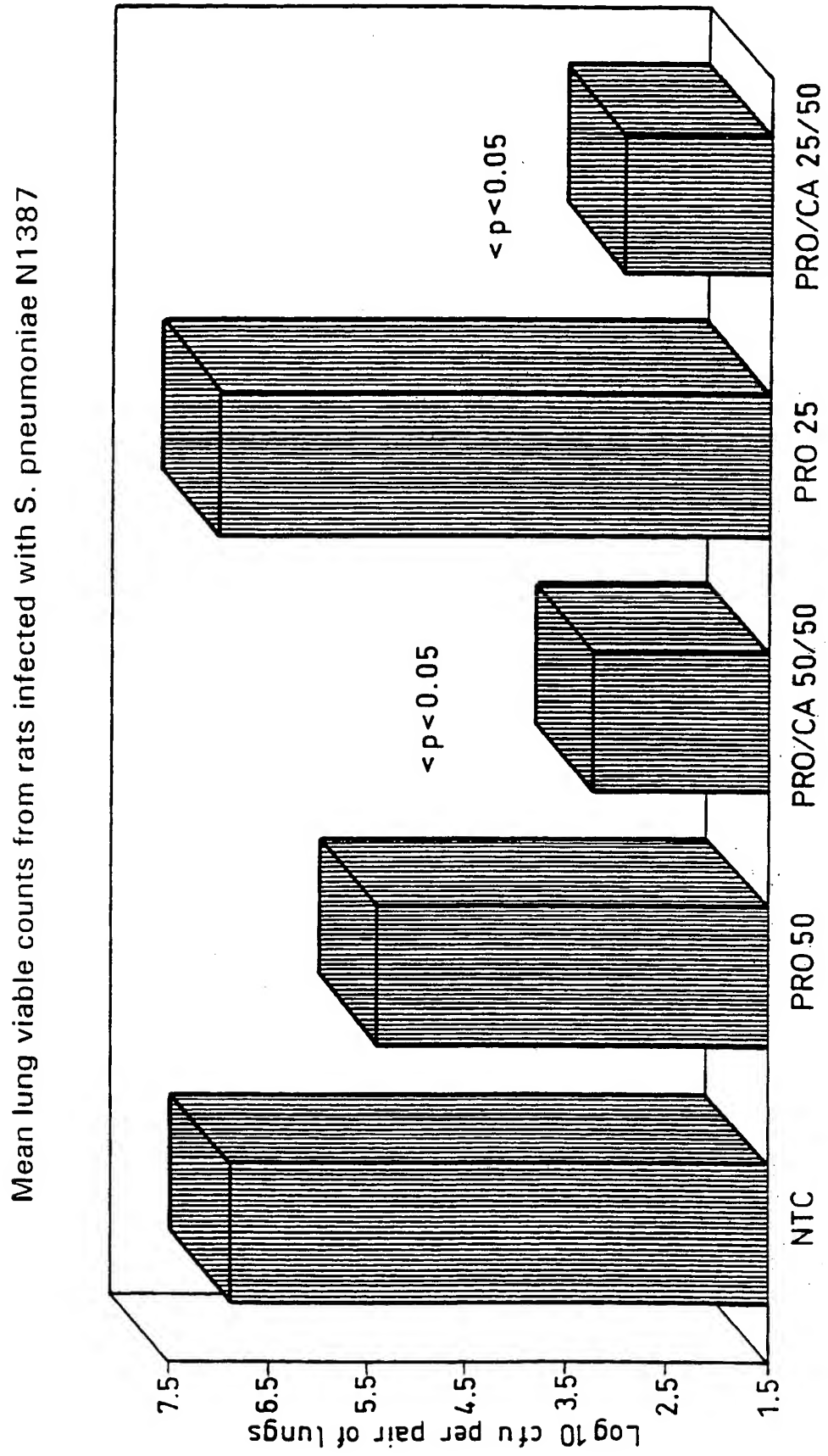
Mean lung viable counts from rats infected with S. pneumoniae N1387
Log10 cfu per pair of lungs
7.5
6.5
5.5
4.5
3.5
2.5
1.5
NTC
PRO 50
PRO/CA 50/50
PRO 25
PRO/CA 25/50
< p < 0.05
< p < 0.05

Fig. 5

INTERNATIONAL SEARCH REPORT

Interna I Application No
PCT/GB 94/00124

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/42 A61K31/545 A61K31/43

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS FIRST SYMPOSIUM AUGMENTIN: EXCERPTA MEDIA INT. CONG. SER. 1980 pages 80 - 83 GREENWOOD, D. 'SHORT COMMUNICATION: A TYPE OF INTERACTION BETWEEN CLAVULANIC ACID AND OTHER BETA-LACTAM ANTIBIOTICS DISTINCT FRON BETA-LACTAMASE INHIBITION EFFECT' cited in the application see the whole document ---	1-11
X	FR,A,2 267 778 (BEECHAM GROUP LTD.) 14 November 1975 see the whole document especially page 11, line 12-36 ---	1-7,9-12
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 May 1994	27.05.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Mair, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna' l Application No
PCT/GB 94/00124

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF CHEMOTHERAPY vol. 5, no. 3 , June 1993 pages 147 - 150 SEGATORE, B. ET AL 'IN VITRO ACTIVITY OF CEFODIZIME (HR-221) IN COMBINATION WITH BETA-LACTAMASE INHIBITORS' see the whole document ---	1-7,10, 15,18, 19,21
A	EUROPEAN JOURNAL OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES vol. 10, no. 2 , February 1991 pages 77 - 84 SUMITA, Y. ET AL 'IN VITRO SYNERGISTIC ACTIVITY BETWEEN MEROPENEM AND OTHER BETA-LACTAMS AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS' -----	1-22

1

INTERNATIONAL SEARCH REPORT

[redacted]ational application No.
PCT/GB94/00124

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1,2,4-14,21 and 22 are directed towards a method of treatment of the human/animal body the search has been carried out and based upon the alleged effects of the compositions.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat l Application No
PCT/GB 94/00124

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
FR-A-2267778	14-11-75	GB-A-	1508977	26-04-78
		AT-B-	350718	11-06-79
		AT-B-	353407	12-11-79
		AT-B-	350719	11-06-79
		AT-B-	348124	25-01-79
		AT-B-	352270	10-09-79
		AU-B-	499171	05-04-79
		AU-A-	8017775	21-10-76
		BE-A-	827926	14-10-75
		CA-A-	1059050	24-07-79
		CA-A-	1055395	15-05-79
		CA-A-	1074325	25-03-80
		CA-A-	1076120	22-04-80
		CH-A-	629252	15-04-82
		CH-A-	630631	30-06-82
		DE-A,C	2517316	23-10-75
		DE-A-	2559410	23-09-76
		DE-A,C	2559411	21-10-76
		DE-C-	2560074	28-03-85
		JP-C-	1054511	23-07-81
		JP-A-	53002494	11-01-78
		JP-B-	55049830	15-12-80
		JP-C-	1054512	23-07-81
		JP-A-	53002495	11-01-78
		JP-B-	55049831	15-12-80
		JP-C-	1054513	23-07-81
		JP-A-	53003588	13-01-78
		JP-B-	55049832	15-12-80
		JP-C-	1054514	23-07-81
		JP-A-	53003589	13-01-78
		JP-B-	55049833	15-12-80
		JP-A-	53003590	13-01-78
		JP-C-	872874	29-07-77
		JP-A-	50142789	17-11-75
		JP-B-	52001996	19-01-77
		JP-C-	916304	15-08-78
		JP-A-	50142591	17-11-75
		JP-B-	52046957	29-11-77
		JP-C-	1008055	31-07-80
		JP-A-	52085189	15-07-77

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna · l Application No
PCT/GB 94/00124

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A-2267778		JP-B- 54043520	20-12-79
		LU-A- 72322	20-08-75
		NL-A- 7504621	22-10-75
		OA-A- 5203	28-02-81
		SE-B- 422063	15-02-82
		SE-B- 435286	17-09-84
		SE-A- 7811200	27-10-78
		SE-B- 446687	06-10-86
		SE-A- 7811201	27-10-78
		SE-B- 435287	17-09-84